HFA-305

JUN 7 1999

FREEDOM OF INFORMATION SUMMARY

Public Master File (PMF) # 5433

Amoxicillin Trihydrate Injection

"...for the treatment of bacterial pneumonia due to *Pasteurella* spp. and *Hemophilus* spp. in sheep."

Sponsored by:

NRSP-7

I. GENERAL INFORMATION

PMF Number:	5433
Sponsor:	NRSP-7 Western Region College of Veterinary Medicine University of California Davis, California 95616
Accepted Name:	Amoxicillin trihydrate
Supplemental Effects:	The approval of a supplement to an already approved product will allow for the use of amoxicillin trihydrate for the treatment of bacterial pneumonia due to *Pasteurella* spp. and *Haemophilus* spp. in sheep.
Minor Species Classification:	Sheep are classified as a minor species. Therefore, this Public Master File addresses minor species requirements with respect to effectiveness and target animal safety data collection.

II. INDICATION FOR USE

Amoxicillin trihydrate is indicated for the treatment of bacterial pneumonia due to *Pasteurella* spp. and *Haemophilus* spp. in sheep.

III. DOSAGE FORM, ROUTE OF ADMINISTRATION, AND DOSAGE

A. *Dosage Form*: Amoxicillin trihydrate injectable (250 mg/mL).

B. *Route of Administration*: Intramuscular or subcutaneous injection

C. *Recommended Dosage*: Administer 10 mg per kg of body weight once daily for up to 5 days. Treatment should be continued for 48 to 72 hours after the animal becomes afebrile or asymptomatic. Do not continue treatment beyond 5 days.

IV. EFFECTIVENESS

Section 514.1(d) of Title 21 of the Code of Federal Regulations (CFR) permits extrapolation of data from a major species to a minor species to satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act with respect to the effectiveness of a new animal drug. A combination of data from sheep (a minor species) and a closely-related approved major species (cattle) were used to support the determination of effectiveness, consistent with the *Guidelines for the Preparation of Data to Satisfy the Requirements of Section 512 of the Act Regarding Minor Use of Animal Drugs* (FDA/CVM April 1986).

A. Type of Study: Pharmacokinetic Serum concentration/time profiles generated after both intravenous (IV) and extravascular (intramuscular, IM, and subcutaneous, SC) administration were used to support the approval of IM and SC amoxicillin injection (10 mg/kg b.i.d.) for the treatment of respiratory infections in sheep.

B. Name and Address of Investigator:

Dr. Mary S. Bulgin,
WOI Regional Program
Caldwell Veterinary Teaching Center
University of Idaho
Route 8, Box 267
Caldwell, Idaho 83605

C. General Design of the Investigation:

PROTOCOLS

Intramuscular (IM) and Subcutaneous (SC) Data

This investigation was conducted as a two-treatment two-period two-sequence crossover. Treatments were separated by a two-week washout period. Five healthy ewes and rams (2 to 5 years of age) were used in this investigation.

A 10 mg/kg dose of the test material (Smith Kline Beecham's Amoxi-Inject® amoxicillin trihydrate, lot #CR7350) was administered via intramuscular (IM) or subcutaneous (SC) injection into the semimembranous muscle or into the dorsum of the neck. All sheep were weighed on the day prior to drug administration. The drug was prepared as a 25% suspension (250 mg/mL) not more than four hours prior to use and was stored at room temperature. Sterile water for reconstitution was maintained at room temperature throughout the study.

Blood was sampled prior to injection and at 5, 10, 15, 30, 45 and 60 minutes and 1.5, 2, 3, 4, 5, 6, 8, 10 and 12 hours post-dose. Serum was assayed for amoxicillin concentrations using a microbiological procedure in which *Bacillus subtilis* is the test organism.

Intravenous (IV) Data

Six healthy 2 to 3 year old Suffolk and crossbred sheep were used in this study. Sodium amoxicillin (Beecham Laboratories) was dissolved in sterile water (48 mg/mL) and was administered as a bolus IV injection (10 mg/kg) into the jugular vein. Blood samples (10 mL each) were taken at 3, 5, 10, 15, 30, 45, 60 minutes and 1.5, 2, 3, 4, 5, 6, 8, 10, 12, and 24 hours post-dose. Serum was separated and frozen at –20 °C. Amoxicillin was assayed using a microbiological procedure whereby *Bacillus subtilis* and a thermospore suspension (*B. sterothermophilus*) served as the test organisms.

Cattle vs Sheep

A 6.6 mg/kg IM dose of the aqueous suspension (100 mg/mL) was administered to several species, including sheep (100 mg/mL) and cattle (250 mg/mL). Jugular vein blood samples were taken at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 14 and 26 hours post-dose. Amoxicillin concentrations were determined by a microbiological assay procedure.

D. Results:

Serum concentrations tended to be somewhat higher after IM dosing as compared to SC administration. Furthermore, concentrations tended to be higher in ewes as compared to rams (Table 4.1). Nevertheless, systemic clearance was similar across all animals (approximately 10 mL/min*kg). The average concentration at the final sampling time, hour 12 post-dose (C_{hr12}) was approximately 5 μg/mL, regardless of route or gender (Figure 4.1).

Table 4.1. Comparison of serum amoxicillin concentration across genders and routes of administration (10 mg/kg).

	C_{hr12}	$AUC_{0\text{-last}}$	CMAX	TMAX
Ewes, IM	0.784^a	12.06^a	1.60^a	0.95
Rams, IM	0.532^b	7.96^b	1.03^b	1.35
Ewes, SC	0.498^b	7.88^b	0.92^b	2.5
Rams, SC	0.474^b	6.64^b	0.75^b	1.75

^{a,b} numbers with like superscripts are not statistically significantly different ($p>0.05$).

The estimated terminal elimination half life following IV injection (approximately 45 minutes) was markedly shorter than that observed following SC or IM administration (8 to 12 hours). Consequently, the decline in serum amoxicillin concentrations following extravascular administration appears to be limited by the rate of drug absorption (flip-flop kinetics).

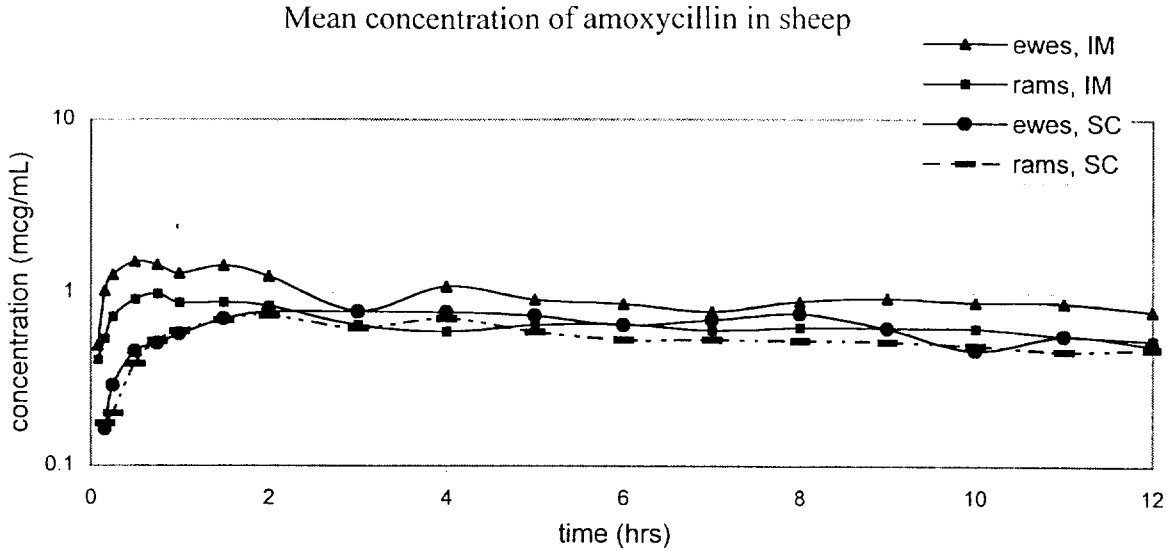

Figure 4.1. Mean amoxicillin concentrations following 10 mg/kg dosages of SKB aqueous suspension.

When comparing the serum concentration/time profiles obtained in sheep versus cattle, no statistically significant differences were observed in AUC or CMAX values. Thus, IM injection of amoxicillin appears to result in similar serum concentration/time profiles for these two species (Figure 4.2).

It should be noted that significant differences in amoxicillin kinetics have been associated with site of drug administration [Rutgers, L.J.E., Van Miert, A.S.P.A.M., Nouws, J.F.M. and Van Ginneken, C.A.M. (1980). Effect of injection site on the bioavailability of amoxicillin trihydrate in dairy cows. *J. Vet. Pharm. Therap.*, **3**: 125-132.]. Accordingly, to maximize the duration of time during which serum amoxicillin concentrations remain above the minimum inhibitory concentration (MIC) of the targeted pathogens, the drug should be administered either into the semi-membranous muscle (IM) or into the dorsum of the neck (SC).

Serum concentration /time profiles in cattle and sheep

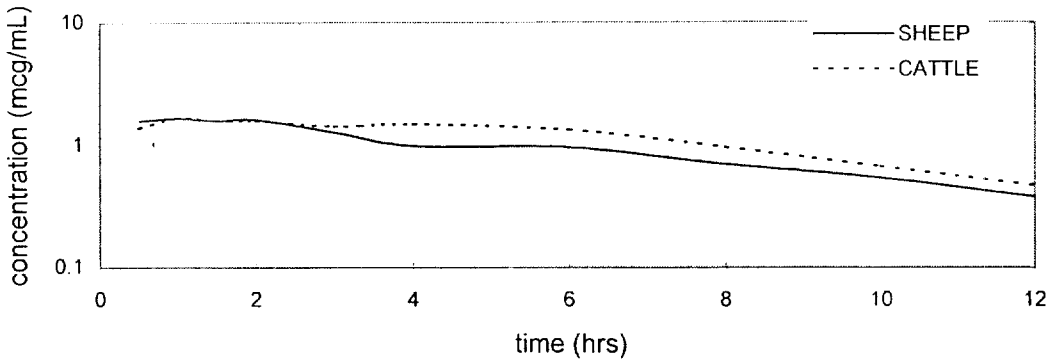

Figure 4.2. Amoxicillin concentrations following 6.6 mg/kg dosages of SKB aqueous suspension.

E. Conclusion:

The data show that amoxicillin trihydrate, when given by the intramuscular or subcutaneous route to sheep provides effective (>0.5 µg/mL) plasma levels for approximately 12 to 16 hours. Thus, amoxicillin trihydrate should be effective in the treatment of bacterial pneumonia due to *Pasteurella* spp. and *Haemophilus* spp. in sheep. Based on the half-life of elimination of amoxicillin in sheep and the bactericidal mechanism of action of amoxicillin, effective treatment regimens would include dosage intervals of up to 24 hours, the same as for cattle.

V. ANIMAL SAFETY

A study demonstrating the safety of amoxicillin trihydrate 25% solution in sheep is summarized below.

A. Type of Study: Target animal safety study

B. Name and Address of Investigators:

Dr. Mary S. Bulgin,
WOI Regional Program
Caldwell Veterinary Teaching Center
University of Idaho
Route 8, Box 267
Caldwell, Idaho 83605

C. General Design of the Investigation:

1) Purpose of the study: To demonstrate that amoxicillin trihydrate is safe for use in sheep.

2) Test Animals: Twenty healthy female crossbred sheep, weighing 30 to 36 kg, were allocated for this study.

3) Treatment groups: The animals were randomly divided into 4 groups of 5 animals each. One group served as a placebo control and received daily intramuscular injections of saline for 5 days, while each of the other 3 groups received a different intramuscular dose of amoxicillin once daily for 5 days.

   Group A - saline at a volume similar to the treatment groups (control).

   Group B - 5 mg/lb/day for 5 days (label dose).

   Group C - 15 mg/lb/day for 5 days (3X).

   Group D – 25 mg/lb/day for 5 days (5X).

4) Dosage Form: Injectable suspension.

5) Route of Administration: Intramuscular.

6) Test Duration: 21 days.

7) Parameters:

   -- daily clinical observations
   – blood chemistries (prior to the initial dose and the day after the last dose)
   -- mortality
   – necropsy findings

D. Results:

No clinical effects were observed in any of the animals, with the exception of transient pain upon injection, particularly in the animals injected with 5 times the recommended dose. No mortality was reported in this study. There were no significant changes in clinical blood chemistry values for any of the groups. Clinical blood chemistries included blood urea nitrogen, glucose, creatinine, uric acid, sodium, potassium, chloride, calcium, phosphorus, iron, total protein, albumin, globulin, alkaline phosphatase, SGOT, total bilirubin, direct bilirubin, indirect bilirubin, cholesterol, triglycerides, and lactic dehydrogenase. No significant lesions were observed during the necropsy of each of the animals in the high dose group (5X). However, there was slight scarring at the injection site of 2 animals. No histopathology studies were performed because no gross lesions were found in any organ system, and there were no significant changes in blood chemistries.

E. Conclusion:

The data demonstrate that amoxicillin trihydrate is safe for sheep at the recommended dose of 5 mg/lb (11 mg/kg) body weight for up to 5 days.

VI. HUMAN FOOD SAFETY

Summary: Tissue Residue Depletion Study in Sheep Treated with Amoxicillin trihydrate

A. Name and Address of Investigators:

A residue depletion study was conducted in sheep injected IM once a day for 5 days with 11 mg amoxicillin/kg body weight. The in life portion of the study was conducted by:

Dr. Mary S. Bulgin,
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Caldwell, Idaho 83605

The analysis of the tissue samples was performed by:

Dr. John G. Babish
Department of Veterinary Pharmacology and Toxicology
College of Veterinary Medicine
University of California
Davis, California 95606

B. Location: University of California, Davis, California

C. Test Animals:

Twenty mixed-breed sheep (10 ewes and 10 rams), weighing between 107 and 335 lbs, were used in the study.

D. Treatment groups:

Nineteen sheep were injected IM once a day for 5 days with 11 mg amoxicillin/kg body weight. One ewe was used as a control.

E. Parameters:

The treated sheep were sacrificed in groups of four (2 males, 2 females) at 3, 10, 15, 18, and 21 days post-dosing. Kidney, liver, fat, skeletal muscle, and injection site samples were collected for analysis. The samples were analyzed using a modified version of the microbiological method used for the amoxicillin injectable for cattle (NADA 55-089). The mean residue values for kidney, muscle, liver, and fat are shown below along with the standard deviations.

F. Results: The results are presented in Table 6.1.

Table 6.1. Mean tissue residue values (ppb) and standard deviations for sheep treated with 5 IM injections of 11 mg amoxicillin per kg body weight.

Withdrawal time in days	Kidney	Liver	Muscle	Fat
3	751 (122)	< 15	< 15	128 (128)
10	32.7 (19)	< 15	< 15	< 15
15	< 15	< 15	< 15	< 15
18	< 15	< 15	< 15	< 15
21	< 15	< 15	< 15	< 15

Limit of quantitation = 15 ppb

G. Conclusions

A statistical analysis using a 99% tolerance limit approach with 95% confidence was performed on the data from the residue study. The results of that analysis indicated that amoxicillin antimicrobial activity in kidney would decline to less than 10 ppb by 21 days postdosing under the conditions of the study.

VII. AGENCY CONCLUSIONS

The data submitted in this public master file (PMF) are supporting information for the effectiveness, target animal safety and human food safety data required by Section 512 of the Federal Food, Drug, and Cosmetic Act with regard to the proposed use of amoxicillin trihydrate injectable for the treatment of bacterial pneumonia due to *Pasteurella* spp. and *Haemophilus* spp. in sheep. Sheep is a minor species of animals defined under 21 CFR 514.1(d)(1)(ii). The data submitted meet the requirements of that regulation, and FDA's "Guidelines for the preparation of Data to Satisfy the Requirements of Section 512 of the Act Regarding Minor Use of Animal Drugs" (April 1986). FDA will consider this information along with other required data as support for NADAs that may be filed for this use of amoxicillin in sheep.

A statistical analysis of data from the residue study using a 99% tolerance limit approach with 95% confidence indicated that residues of amoxicillin in sheep kidney would decline to less than 10 ppb by 21 days post-dosing under the conditions of the study.

FDA is publishing a notice of availability of the PMF to encourage sponsors to file new animal drug applications (NADAs) for amoxicillin trihydrate for the use covered by this PMF. Sponsors will need to include in their application, in addition to a reference to this PMF, drug labeling and other information needed for approval, such as data supporting extrapolation from a major species in which the drug is currently approved, or authorized reference to such data, and data concerning manufacturing methods, facilities, and controls, and information addressing the potential environmental impacts (including occupational) of the manufacturing process associated with the product.

Amoxicillin trihydrate is currently approved for use by IM or SC injection at a dosage of 3 to 5 mg per pound of body weight once daily for up to 5 days for the treatment of respiratory infections due to *Pasteurella multocida*, *P. haemolytica*, *Haemophilus* spp., *Staphylococcus* spp., and *Streptococcus* spp. and acute necrotic pododermatitis due to *Fusobacterium necrophorum* in cattle. See 21 CFR 522.88.